single or double unit recipient groups. There was no significant difference in overall survival. However, when comparing patients that survived >9 mo after transplant there were significant differences. No patient in the double UCB group experienced further events, however, in the single UCB group, there were 15 events, 13 of which were due to disease recurrence ($p<0.01$). Collectively, these data support the concept that the infusion of two partially HLA matched UCB units results in rapid engraftment, a higher rate of aGVHD and a lower rate of leukemia recurrence. We hypothesize that the co-infusion of two, partially HLA matched UCB units leads to increased alloreactivity and this accounts for the above findings.

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A NOVEL TRIPLE UMBILICAL CORD BLOOD TRANSPLANT (UCBT) STRATEGY TO PROMOTE NK CELL IMMUNOTHERAPY (UNIT I) WITH A FULLY ABLATIVE PREPARATIVE REGIMEN FOLLOWED BY A DOUBLE UCBT IN PATIENTS WITH REFRACTORY AML

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Previously, we tested adoptive transfer of haploidentical peripheral blood (PB) derived NK cells without transplantation in patients with refractory AML and demonstrated a correlation between in vivo NK cell expansion and complete remission. This therapy is limited by: 1) the inability to expand NK cells in most patients, 2) prolonged neutropenia and 3) inconsistent efficacy. We hypothesized that UCB-derived NK cells may show better in vivo expansion because UCB is rich in NK cell precursors. Therefore, we tested our triple UCBT strategy in patients with refractory relapsed AML. UCB-derived NK cells (matched at 3 HLA loci and KIR-ligand mismatched when possible) were infused (after CD3 depletion and IL-2 activation) on day –12 after conditioning with cyclophosphamide, fludarabine and TBI. Subcutaneous IL-2 (10 MU $\times$ 6) was given over 10 days to facilitate in vivo NK cell expansion. On day 0, two UCB units ($\geq$ 4/6 match) were transplanted (DCBT). Ten patients have been treated and unexpectedly, four patients exhibited neutrophil engraftment from the processed and IL-2 activated NK cell Unit #1. One patient developed cyclophosphamide induced cardiac failure and died prior to DCBT. All others tolerated the NK infusion and IL-2 without unexpected toxicity and were leukemia-free at the time of engraftment. Two remain alive and disease free at 3+ and 8+ months and three died disease free of TRM. Four have relapsed after initially clearing their leukemia. These data suggest UCB NK cells may be administered safely and, despite CD3 depletion and IL-2 activation (ex vivo and in vivo), provide long-term engraftment potential that may dominate over unmanipulated UCB infused subsequently. In summary, UCB is a rich source of NK precursors capable of in vivo expansion which are potentially better suited than adult NK cells for use in treatment of patients with refractory AML.

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EX-VIVO EXPANSION AND MRNA TRANSFECTION OF CORD BLOOD DERIVED NATURAL KILLER CELLS

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Natural Killer (NK) cell-mediated alloreactivity can control leukemia relapse while protecting patients from graft-versus-host disease (GVHD) after allogeneic stem cell transplant. Cord blood (CB) is rich in NK cell progenitors and represents an attractive platform for cytotoxic cell therapy. Our objective was to *ex-vivo* expand cord blood NK cells followed by genetic modification to ultimately render them cancer cell specific by introducing chimeric antigen receptors. CB mononuclear cells were cryopreserved after CD3 depletion performed by immunomagnetic microbead selection (Miltenyi Biotec, Auburn, CA). Cells were plated for NK expansion, with or without a feeder layer of irradiated umbilical cord mesenchymal cells (UC-MSC) obtained from the Wharton's jelly either from the same (autologous) or from an unrelated (allogeneic) cord donor, with or without IL-2 (1000 IU/ml), IL-15 (10 ng/ml), IL-3 (10ng/ml) and Flt3 (10ng/ml). At a median of 19 days of culture (range 14-21), there was significantly higher expansion (range 3.5-72 fold) of $CD56^+/CD3^-$ cells with the UC-MSC feeder layer and cytokines compared to controls (mean 21.2 $\pm$ 20.8 fold increase vs 1.6 $\pm$ 0.9 fold increase with feeder layer only and 1.8 $\pm$ 0.89 fold increase with cytokines only, $p=0.039$ and $p=0.041$ respectively). There was no significant difference in NK expansion between autologous and allogeneic UC-MSC feeder layers (29.6 $\pm$ 26.8 vs. 12.8 $\pm$ 8.9 fold, $p=0.243$). NK cells expansion was directly proportional to the number of UC-MSC plated as feeders. Expanded CB-NK cells also displayed enhanced cytotoxicity compared to control cultures plated with cytokines only (91.78$\pm$0.7% vs. 82.5$\pm$1.8%, $p=0.003$ and 89$\pm$2.3% vs. 83.7$\pm$0.18%, $p=0.056$, respectively). To test whether expanded CB-NK cells can be genetically engineered for ultimately targeting malignant cells, we transfected via electroporation (Bio-Rad) expanded CB-NK cells with mRNA produced from plasmid GFP-cDNA. GFP-mRNA expression (flow cytometry) at 24 hours was significantly higher (mean 42.8$\pm$5.2%) compared to GFP-cDNA controls (mean 4.2$\pm$0.35%, $p<0.001$). Mean GFP-mRNA expression was 35%, 31% and 16.5% at 48, 72 and 144 hours respectively. In summary, CB-NK cells can be effectively expanded on a feeder layer of UC-MSC while preserving cytotoxicity and can also be genetically engineered by mRNA transfection.

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UPDATE ON THE C.W. BILL YOUNG CELL TRANSPLANTATION PROGRAM & NATIONAL CORD BLOOD INVENTORY

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The Stem Cell Therapeutic and Research Act of 2005 (Public Law 109-129) was signed by President Bush on December 20, 2005. Administrative responsibility for most provisions of the Act is assigned to the Health Resources and Services Administration (HRSA) within the Department of Health and Human Services (HHS). Section 2 of the Act authorizes Federal support for collection and maintenance of 150,000 new, high quality cord blood units for a National Cord Blood Inventory (NCBI), authorizes a related cord blood donor demonstration project, and requires that some cord blood units not suitable for clinical transplantation be made available for research. Contracts for NCBI collections are awarded based on technical merit, the ability to recruit and collect cord blood units from diverse populations, bank location (to ensure geographic dispersion), and price. Contracted banks must also demonstrate the ability to assist during catastrophic events that may result in an increased use of umbilical cord blood for hematopoietic reconstitution. The six umbilical cord blood banks awarded contracts in November, 2006 (first cohort) are: Duke University Medical Center and Health Systems; the University of Texas M.D. Anderson Cancer Center; the New York Blood Center; the Puget Sound Blood Center; StemCyte, Inc.; and, the University of Colorado at Denver Health and Sciences. Of the cord blood units collected and banked with the monies awarded in 2006, approximately 63% will be from racial and ethnic minorities. HRSA has begun the competitive contracting process for a second round of awards to cord blood banks (second cohort). Implementation of the related cord blood donor demonstration project has begun with the publication of a request for information on HRSA's proposed approach in the spring of 2007. Section 3 of the Act authorizes the C.W. Bill Young Cell Transplantation Program (successor to the National Bone Marrow Donor Registry). Among other functions, the C.W. Bill Young Cell Transplantation Program (Program) is charged with creating a single point of access